EXHIBIT 603.20

The Nightmare of Postmortem Drug Changes

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The purpose of postmortem analysis for drugs is to determine as accurately as possible the concentration of the drugs that existed in blood at the time of death, in order to assess the likelihood of drug toxicity, and, in particular, whether the death can be explained by the drug concentration found. Drug and metabolite concentrations in blood are interpreted by comparison with previously reported concentrations corresponding to therapeutic, toxic, and fatal conditions. In all of this there is an underlying presumption that drug concentrations in blood, and other biological fluids and tissues, remain constant in a corpse whatever the delay between death and the collection of samples. In recent years it has become increasingly clear that, for most drugs, this presumption is false. Blood drug concentrations do change significantly during the postmortem period.

The idea is not entirely new and there have been indications of unease in occasional papers over the past thirty years. In 1960[1] it was noted that concentrations of barbiturates in blood obtained from vessels within the central body cavities were significantly different to blood obtained from femoral vessels. This was later confirmed[2] and similar observations reported for acetaminophen[3] and digoxin.[4,5] By the early 1980s it was clear that this problem also involved propoxyphene and the tricyclic antidepressants amitriptyline, nortriptyline, desipramine, and doxepin,[6,7] and it was hypothesized that the postmortem release of drugs sequestered in the liver during life resulted in a rise in blood drug concentration which increased with the postmortem interval and varied with blood sampling site. We now know that this is true but that it is only one facet of highly complex changes in blood and tissue drug concentrations that occur postmortem.

Before discussing postmortem drug changes, it is important to recognize that site dependent differences in blood drug concentrations can arise antemortem. During drug absorption, there is distribution of the drug from the blood to the tissues and this distribution phase lasts approximately thirty minutes to two hours for most drugs.[8] During this period there can be a sizeable difference between arterial and venous drug concentrations and this might be reflected in site differences in postmortem blood drug concentrations where a person has died during the absorptive phase. In a dog model of this situation,[9] amitriptyline was administered directly into the duodenum at doses of 80, 250, and 500 mg/kg to simulate acute overdose in humans. Femoral artery, femoral vein, carotid artery, and jugular vein blood samples were drawn over a three-hour period or until the death of the animal. There were significant variations in the concentration of amitryptyline and nortriptyline between the sampling sites, for all three drug doses. At higher drug doses the concentration in arterial blood was sometimes as much as twice that of venous blood.

TABLE 1

Femoral Artery and Vein Plasma Concentrations (μmol/L) of Amitriptyline in Clinical Intoxications (Abstracted from Table 2, Reference 10)

Patient	Intoxication /admission interval (h)	Plasma conc (μmol/L) Arterial	Venous	AVD	Extraction coefficient (%)
2	—	3.5	4.75	−1.25	−35.7
4	—	1.92	2.40	−0.48	−25
6	6	2.80	2.00	+0.80	+28.6
9	19	2.27	1.70	+0.57	+25.1
10	2	3.27	1.45	+1.82	+55.7
13	4	2.62	1.34	+1.28	+48.8
17	10	1.90	0.82	+1.08	+56.8
18	14	0.58	0.80	−0.22	−37.9
19	1.5	1.1	0.78	+0.32	+29.1
20	2	0.94	0.64	+0.30	+31.9
23	3	2.05	0.47	+1.58	+77.1
24	5	0.50	0.23	+0.27	+54.0

AVD = arterio-venous difference
extraction coefficient = the ratio of the arterio-venous difference to the arterial plasma concentration

Studies of arterio-venous differences in drug concentration in humans are scarce. A clinical study[10] demonstrated significant arterio-venous differences in amitriptyline concentration early in the course of acute intoxication. The study group of twenty-four comatose patients (fourteen women and ten men, age range eighteen to sixty-six years) had a mean delay between ingestion and blood sampling of 7.5 +/− 5 hours (range 1.5 to 19 hours). Blood samples drawn simultaneously from the femoral artery and vein gave venous plasma concentrations of amitriptyline within the therapeutic range (0.3 to 1 μmol/L) in ten patients in contrast to arterial plasma concentrations which were above the therapeutic range in four. Similarly in the nine patients considered to be only mildly poisoned according to venous concentrations (1 to 2 μmol/L), arterial concentrations were above 2 μmol/L in five. It was concluded that arterial plasma concentrations of amitriptyline are likely to be higher than the venous concentrations during the absorption-distribution phase due to uptake of the drug by the extravascular tissues. Some of the original case data is set out in Table 1. This phenomenon may be one factor influencing postmortem blood drug concentration differences in acute drug overdoses but alone cannot

fully explain all the published observations, particularly blood drug concentration changes with increasing postmortem interval.

Studies on postmortem drug concentration changes in blood typically involve comparisons between samples from different sites. Two general problems that arise in making such comparisons are the dyshomogeneity of blood samples due to postmortem clotting and the loose attribution of samples to particular anatomical sites.[11]

The loose attribution of blood samples to particular anatomical sites is a general problem both in case work and in the forensic literature. Blood samples may be obtained by "blind" needling of the body, or by techniques improvised on a case-by-case basis by the individual pathologist, or by a technique which is likely to result in a false attribution of site of origin. Blind needling of the chest to produce a "cardiac sample" may give a sample from any of the cardiac chambers or great vessels, and experience shows that drug concentrations can vary several fold between these sites. Such a blood sample is really a thoracic sample of unknown anatomical provenance and should be viewed as such. Also autopsy blood sampling techniques vary considerably from pathologist to pathologist and may be improvised on a case-by-case basis; it is uncommon for there to be a written, adhered to, detailed sampling protocol. Consequently a "cardiac sample" may mean one obtained by needle puncture or incision of a specific, or any, cardiac chamber or alternatively pooled blood from the pericardial sac after excision of the heart, a practice best avoided. A sample labeled simply "blood" may differ in origin from case to case as the pathologist varies sampling technique according to the exingencies of the moment, which pass undocumented.

Even blood samples of clearly stated provenance may have a different anatomical origin because of flaws in the sampling technique. It is common practice for femoral blood to be sampled without cross-clamping or tying off the vessel so that there is a resulting high risk of drawing blood from the contiguous, larger iliac vein and the inferior vena cava rather than the smaller peripheral vessel. Similarly so-called subclavian blood is commonly drawn without tying off the vessel and from a point midway between the shoulder and the sternum. The sample obtained in this manner is likely to be drawn largely from the superior vena cava and the right atrium and may therefore be poorly representative of peripheral blood. Consecutive blood samples drawn from a single site will not necessarily be homogeneous because later samples are almost certainly siphoned from contiguous vessels. Eight consecutive aortic blood samples taken at the autopsy of a twenty-one-year-old psychiatric inpatient illustrate this with respect to tricyclic antidepressants. The drug concentrations in the successive samples were 0.4, 0.5, 1.8, 2.0, 2.9, 3.3, 3.2, and 3.3 mg/L.[12] Ideally a blood sample should be obtained by needle puncture of the vessel after cross clamping or ligating it.

It is well established that some drugs may be unevenly distributed between plasma and red cells in blood. Since postmortem blood sediments and clots unevenly in the body, this might also account for a small proportion of the site-to-site blood drug concentration differences observed in case studies. When a

postmortem examination is performed more than an hour or so after death, the blood in the vessels may be clotted, or completely fluid, or partly clotted and partly fluid. If the blood is fluid it is also incoagulable. Studies, both in vitro and in mortuo, have provided some understanding of this phenomenon.[13] The transformation is brought about by clotting of the blood followed by lysis of the clot. The two processes occur simultaneously and the effectiveness of the clot lysis will determine whether the blood at autopsy is clotted, or completely fluid, or partly clotted and partly fluid. This is quite variable from case to case. When fibrin clot is present it always entraps large numbers of red blood cells so that the resultant clot is relatively red cell rich. Sampling of these clots for toxicological analysis will clearly influence the detected concentration of any drug that has an unequal distribution between red blood cells and serum. The blood obtained from limb vessels is most likely to be fluid and largely devoid of clots, reflecting the approximately inverse relationship between the endothelial-derived fibrinolytic activity and the diameter of the vessel from which the blood was obtained.[13] Thus uncoagulable fluid blood often, but not universally, present in the limb vessels provides as homogeneous a sample for analysis as can be hoped for. Even so, the problem of the lack of homogeneity of postmortem blood does not appear to be a major one. Thus far,[11] there is no proved correlation between the differences in the hemoglobin concentration of postmortem blood samples and differences in the concentrations of drugs such as imipramine and desipramine. Certainly a lack of sample homogeneity cannot explain the more extreme site-to-site differences in blood drug concentrations which have been documented.

In fatalities from an oral drug overdose it is commonly assumed, although not usually known with certainty, that the drug was taken as one single large dose. The rupturing of the drug-filled condom in the stomach is an uncommon circumstance where there is anatomical proof of a single large oral dose. In one such case[14] a condom containing barbiturates ruptured in the stomach of a thirty-eight-year-old male who came to autopsy an estimated thirty-six hours after death. Toxicological studies on eleven anatomically distinct blood samples disclosed the presence of amobarbital, secobarbital, and pentobarbital in concentrations ranging from 4.3 to 25.8 mg/L, 3.9 to 25.3 mg/L, and 5.1 to 31.5 mg/L, respectively. Highest concentrations were found in the suprarenal portion of the inferior vena cava and lowest concentrations in the subclavian and femoral venous samples. Such a wide range of variation in postmortem blood drug levels is relatively common.

One medicolegal jurisdiction[15] had an established system whereby blood samples were taken at the time of discovery of the body and again at the later autopsy. These time-separated samples provide a useful broad database involving seventeen drugs. In general the drug concentration in the autopsy cardiac sample was higher than in the initial cardiac blood sample taken at the scene of death. However autopsy femoral blood drug concentrations were generally the same as or greater than the initial cardiac sample, but the magnitude of the increase was much less than that seen in the autopsy cardiac sample. Thus drug concentrations

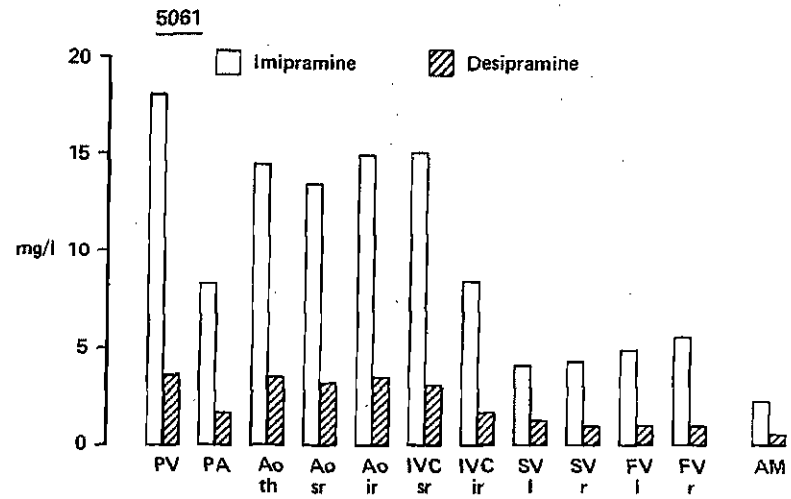

FIGURE 1
Concentrations of Imipramine and Its Metabolite Desipramine in Multiple Blood Samples
PV = pulmonary vein
PA = pulmonary artery
Ao th = thoracic aorta
Ao sr = suprarenal part of abdominal aorta
Ao ir = infrarenal part of abdominal aorta
IVC sr = suprarenal part of inferior vena cava
IVC ir = infrarenal part of inferior vena cava
SV = subclavian vein
FV = femoral vein
AM = ante mortem sample

in cardiac and femoral blood increased postmortem, but the change was more dramatic in cardiac blood.

One of many published case examples in which postmortem blood drug concentrations can be compared with an antemortem sample was that of a forty-seven-year-old man who took a suicidal overdose of the tricyclic antidepressant imipramine.[14] Autopsy was performed 9.25 hours after the taking of an emergency room blood sample and 7.25 hours after death. Imipramine and desipramine concentrations in the antemortem blood were 2.3 mg/L and 0.6 mg/L, respectively; concentrations in eleven postmortem blood samples (obtained after vessel ligation) ranged from 4.1 to 18.1 mg/L and 1.0 to 3.6 mg/L, respectively. Lowest concentrations were found in the subclavian and femoral veins, high concentrations in the aorta and suprarenal portion of the inferior vena cava, and the highest concentration in the pulmonary vein (Figure 1). The drug and its metabolite were

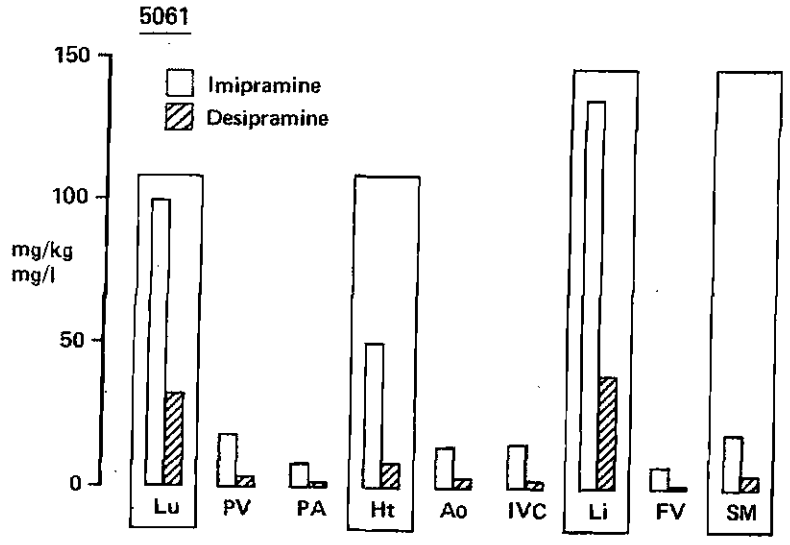

FIGURE 2
Concentrations of Imipramine and Its Metabolite Desipramine in Tissues and Blood from Related Vessels
Lu = lung
Ht = heart
Li = liver
SM = skeletal muscle

most heavily concentrated in the liver (imipramine 136 mg/kg, desipramine 39 mg/kg) and lung (100 mg/kg, 34 mg/kg) and least concentrated in skeletal muscle (16 to 24 mg/kg, 3.8 to 6.7 mg/kg), so that relative concentrations in solid organs broadly paralleled relative concentrations in blood from their associated vessels (Figure 2). The high drug concentrations in the inferior vena cava and pulmonary vein reflect postmortem diffusion of the drug from the liver and lung respectively.

In contrast to the abdominal and thoracic vessels, the peripheral arteries and veins of the limbs are relatively protected by distance from drug release from the major organs. Provided that a drug is not preferentially concentrated in skeletal muscle at many times the blood concentration, then theoretically peripheral blood drug concentration should be more closely representative of, although not necessarily the same as, the drug's concentration at the time of death.

Recently detailed postmortem studies have been undertaken where multiple blood samples have been obtained from different sites, sometimes at repeated time intervals. The enormous expenditure of time and labor involved in such case studies has necessarily meant that their number is small. These studies have

clearly established that drug concentrations in blood samples are dependent on the sampling site and that there is a tendency for the drug concentration to increase with time. However it is difficult to discern a clear pattern that would allow a prediction as to which specimen would have the highest drug concentration or which specimen would demonstrate the most dramatic change in concentration with time.[16] The changes have been observed in cases that involve both therapeutic and overdose ingestions of drugs.

One extremely detailed case study was undertaken on a twenty-five-year-old female who died from a suicidal overdose of imipramine, acetaminophen, codeine, diphenhydramine, and ethanol.[11] Ten blood samples, twenty-four tissue samples, cerebro-spinal fluid, vitreous humour, and bile were analyzed. Concentrations of imipramine in the ten blood samples differed by as much as 760 percent (range 2.1 to 16.0 mg/L) and desipramine concentrations ranged from 1.4 to 10.6 mg/L, diphenhydramine ranged from 0.34 to 2.07 mg/L, and codeine from 0.33 to 0.89 mg/L, but concentrations of acetaminophen differed by less than 20 percent (55 to 65 mg/L) and blood ethanol concentrations ranged from 151 to 175 mg/100ml. The data from this case illustrate well the point that a marked site dependent variability in postmortem blood concentration exists for some drugs but not others. The drugs with the widest concentration ranges in blood (imipramine and desipramine) were also those most highly concentrated in the solid organs and tissues, particularly the lungs and the liver, that is, those drugs with the higher volumes of distribution.

The volume of distribution (Vd) is an important pharmacokinetic concept. The Vd is a theoretical volume that does not correspond to any physiologic space. It is the hypothetical volume of body fluid that would be necessary if the total amount of drug in the body were distributed at the same concentration as in plasma. The Vd is expressed as litres per kilogram of body weight. For a drug that distributes to plasma only the Vd approximates 40 mL/kg. A Vd of 160 mL/kg implies a drug with extracellular distribution only. For a drug that has total body water distribution and can enter cells, for example, ethanol, the Vd approximates 640 mL/kg. Some drugs have an apparent Vd greater than that of total body water and with these drugs tissue depots sequester the high drug concentrations. The Vds of several commonly encountered drugs are set out in Table 2. If a drug has a high Vd then this indicates that it has the potential to exhibit postmortem diffusion from tissue depots into blood. Most drugs of abuse are lipophilic, organic bases which are known to concentrate, to a considerable extent, in solid organs thus providing a gradient for passive diffusion after death.

Compound drug preparations in which two or more drugs are incorporated into the same tablet allow comparison of the postmortem behavior of drugs known to have been taken simultaneously. One example of such a compound preparation is co-proxamol (British Approved Name) which comprises dextropropoxyphene hydrochloride and paracetamol in the mass proportions one part: 10 parts (32.5 mg:325 mg). The two drugs have significantly different pharmacokinetic character-

TABLE 2

Volumes of Distribution of Some Commonly Encountered Drugs

Drug	Vd(L/kg)	Drug	Vd(L/kg)
Amitriptyline	18–22	Imipramine	11–16
Amphetamine	3–5	LSD	0.27
Aspirin	0.15	Methadone	5
Caffeine	1	Methylphenidate	11–33
Chlordiazepoxide	0.26–0.58	Morphine	3.2 (i.v.)
Cocaine	1.2–1.9	Paracetamol (Acetaminophen)	0.75–1
Codeine	5	Pentazocine	4.9
(Dextro)propoxyphene	10–18	Pentobarbital	0.5–1
Diazepam	0.95–2	Phenylcyclidine (PCP)	5.6–6.8
Digitoxin	0.54	Thiopental	1.4–6.7
Digoxin	5–7	Trazadone	0.89–1.5
Dihydrocodeine	1.28 (i.v.)	Triazolam	0.8–1.3
Diphenhydramine	3–7	Trimipramine	20–50

i.v. = after intravenous dose

istics. Dextropropoxyphene has a large Vd (10 to 18 L/kg), a high degree of protein binding, and a high organ concentration. By contrast, paracetamol has a Vd of approximately 0.75 to 1 L/kg, is up to 50 percent plasma protein bound at toxic concentrations, and shows little organ concentration. The contrasting behavior of these two drugs is well illustrated by the detailed analytical data from four co-proxamol case fatalities.[17] Dextropropoxyphene shows site dependence that is much more marked and more variable than paracetamol (Table 3).

The value of the Vd of a drug in predicting its likely tendency to show postmortem drug redistribution is illustrated by the postmortem toxicokinetics of trazodone,[18] a structurally unique bicyclic antidepressant. In two case fatalities respectively, blood trazodone concentrations in ten initial autopsy blood samples ranged from 13.7 to 17.3 and 14.4 to 16.9 mg/L. Twenty-four and forty-eight hours later the respective ranges were 12.8 to 18 and 12.4 to 19.1 for the first case, and 12.5 to 20.1 and 12.7 to 27.0 for the second case. This suggests that blood trazodone concentrations are relatively stable with a less than 40 percent increase sixty hours postmortem and a less than two-fold change during early putrefaction. The volume of distribution of trazodone ranges from 0.89 to 1.5 L/kg, and with such a relatively low Vd, trazodone would not be expected to show particularly marked preferential concentration in solid organs, as was borne out by the case data. Lacking significant solid organ depots of drug, trazodone had less potential for postmortem redistribution than a typical tricyclic antidepressant. Fortuitously, for comparative purposes the first case fatality had trimipramine blood concentrations at zero time, twenty-four hours and forty-eight hours ranging

TABLE 3

Drug Concentrations (mg/L) in Autopsy Blood Samples from Different Sites in Four Cases of Co-proxamol Overdose

	Paracetamol (mg/L)				Propoxyphene (mg/L)			
Site	1	2	3	4	1	2	3	4
Peripheral	361	244	249	277	3.1	1.4	3.5	4.0
IVC-ir	464	242	548	338	4.6	2.0	17.9	5.5
IVC-sr	498	329	687	360	4.0	6.7	32.3	5.0
SVC	468	247	386	—	12.8	5.8	14.1	—
PA	427	261	619	—	10.8	6.9	19.9	—
PV	484	332	530	—	22.2	14.4	27.7	—
Left heart				307				3.5
Right heart				361				5.0
Aorta	439	321	1142	—	4.1	8.8	48.0	—
Portal vein	802	337	—	—	35.0	20.7	—	—

IVC-ir = inferior vena cava, infrarenal
IVC-sr = inferior vena cava, suprarenal
SVC = superior vena cava
PA = pulmonary artery
PV = pulmonary vein

from 5.5 to 11.4, 5.2 to 14.3, and 4.2 to 18.2 mg/L, respectively. Trimipramine has pharmacokinetic characteristics typical of tricyclic antidepressants with a Vd ranging from 20 to 50 L/kg reflecting marked concentration in solid organs, particularly liver, and thus potential for significant postmortem redistribution.

Tricyclic antidepressants are commonly encountered postmortem. They probably represent the most common life threatening therapeutic drug ingestion worldwide. It is not surprising that they should be taken frequently in overdose because they are prescribed for a condition that is associated with a high incidence of self-poisoning and successful suicide. A typical therapeutic dosage of a tricyclic antidepressant (TCA) is 2 to 4 mg/kg/day while 15 to 20 mg/kg is thought to be potentially lethal.[19] Doses in excess of 1 g in an adult are likely to cause moderate to severe symptoms, and doses in excess of 2 g almost always cause severe complications and may well prove fatal.[20] It has been suggested[21] that blood concentrations (sampling site not stated) of 0.5 mg/L or less would not be expected to be associated with toxicity; concentrations of 0.5 to 1.0 mg/L may result in some toxicity but would not be expected to be fatal; concentrations above 1.0 mg/L are clearly toxic and generally fatal, while concentrations above 6.0 mg/L are clearly fatal. The large liver to blood concentration ratios of TCAs can result in postmortem tissue release giving rise to an elevated blood TCA level, and this is true for therapeutic and overdose ingestions.[22] It has been shown that "subclavian vein blood" TCA concentration can increase from less than 0.5

mg/L to more than 1.5 mg/L, creating the potential for falsely implicating TCAs as the cause of death.[23]

Furthermore, there is great individual variation in plasma concentrations of TCAs following therapeutic doses. Plasma levels are not used clinically to adjust dosage, and often patients receiving chronic therapeutic doses have elevated plasma concentrations in the same range as observed after overdose. Consequently using TCA plasma levels alone to distinguish between an acute overdose and a chronic therapeutic dose is difficult because of the marked overlap in plasma concentrations in these two situations. It has been suggested[24] that the parent drug to metabolite ratio may be more helpful in diagnosis: a ratio greater than 2 suggesting an acute overdose while a ratio less than 2 being more consistent with high steady-state plasma levels following therapeutic dosages. However, a significant minority of overdose cases have ratios below 2 and a significant minority of chronic therapeutic cases have ratios above 2, so that plasma parent drug to metabolite ratio is not an absolute indicator. Others[23] suggest that liver TCA concentrations and liver parent drug to major metabolite ratio may aid in distinguishing therapeutic from overdose ingestions, but similar caveats apply. It is worth noting that in clinical management, tricyclic overdoses cannot be judged as to their seriousness simply by evaluating the plasma levels, unlike many other drug poisonings (e.g., barbiturate, ethanol, and salicylate), where drug levels represent a cornerstone of assessment. It seems rash to adopt the view of one author[25] that parent TCA blood concentrations that exceed 1 mg/L may be regarded confidently as a cause of death when no other cause is found at autopsy.

The postmortem elevation of drug concentrations in blood is the result of diffusion from depots in solid organs. To permit this postmortem diffusion process, factors must come into play that allow for the release of the drugs from their binding sites in the solid organs. These are likely to be complex, physicochemical changes occurring as part of the processes of autolysis and, later, putrefaction. Changes in pH, the tissue binding characteristics for the drug, and cell membrane integrity are all probably elements. Cell death itself will bring to an end any energy-dependent drug concentrating systems. The loss of cell membrane integrity is paralleled by the release of intracellular enzymes from the solid organs into the blood. It is this phenomenon that results in the artefactual postmortem elevation of cardiac myocyte and hepatocellular enzymes.[26] If such large molecules as these enzymes pass rapidly into the blood postmortem, then it is hardly surprising that much smaller drug molecules do so. It may be that in the future the assessment of enzyme levels in blood samples will prove to be one practical method of gauging the likely extent of collateral drug release. A simple experimental model of drug release from excised liver fragments[27] offers some encouragement in this.

Postmortem changes in extracellular and intracellular pH may have an important influence on drug redistribution. Drugs are weak acids or bases and in solution they become ionized when they lose or gain a hydrogen ion. The degree of ionization of a compound depends on both its pKa and on the pH of the

solution in which it is dissolved, a relationship described by the Henderson-Hasselbalch equation. Since the lipid soluble form (non-ionized) of a weak electrolyte is the species that crosses the cell membranes, organic acids are more likely to diffuse across membranes when they are in an acid environment whereas a basic environment favors diffusion of bases across membranes.[28] In this way drugs become trapped in the compartment in which they are more ionized because ionized (polar) compounds do not easily cross cell membranes. Most drugs enter cells by crossing the cell membrane by simple diffusion. Intracellular fluid is more acidic than extracellular fluid at physiological pH during life, and consequently bases cross the cell membrane and are trapped in the intracellular compartment. One consequence of this relative partition is a higher Vd. However, there is a sharp decrease in blood pH immediately after death as a result of continuing cellular metabolism with carbon dioxide accumulation until available oxygen is exhausted and then anaerobic metabolism of glucose to lactic and pyruvic acids. In rats the premortem blood pH of 7.34 (SD = 0.02) fell to 6.74 (SD = 0.05) 5 minutes after death and in eleven human fatalities the left cardiac blood pH ranged from 6.64 to 5.60 at between 2 and 20.4 hours postmortem.[29]

The very rapid changes in blood pH that occur postmortem may be responsible for the equally rapid redistribution of some drugs. For example morphine is an amphoteric compound and becomes increasingly lipophilic with increased pH. Morphine is lipid soluble at physiologic pH, but tends to maintain some water solubility over a wide pH range. In a rat model[30] the immediate effect of death included acidosis and significant increases in blood morphine concentration that were evident as early as five minutes after death. Such a rapid increase can be explained by increased partitioning of the drug into the blood from lipid-rich depots in tissue related to acidosis. In the later postmortem period tissues that had the lowest pH, namely cardiac blood (pH 5.55 at 96 hours), heart and liver, also showed the greatest increase in morphine concentration. The authors hypothesized that late postmortem morphine redistribution is the product of morphine glucuronide hydrolysis followed by partitioning of free morphine to tissues based on relative water solubility at equilibrium.

In the forensic literature, attention on the solid organs responsible for postmortem drug redistribution has tended to focus on the liver at the expense of the lungs. However, diffusion of drugs from depots in the lungs into the pulmonary circulation appears to occur more rapidly and to a greater extent that from the liver into the inferior vena cava.[17] The lungs receive the entire right ventricular output and therefore drug distribution into and accumulation in this tissue is very rapid in vivo. Compounds that accumulate in the lungs are usually basic amines, for example, imipramine, amphetamines, methadone, and chlorpromazine, that possess large lipophilic moieties and have pKa values greater than 8.0. The same carrier-mediated sodium-dependent transport systems which remove 5-hydroxytryptamine and norepinephrine from the pulmonary circulation are thought to be responsible also for the removal of exogenous basis amines. For

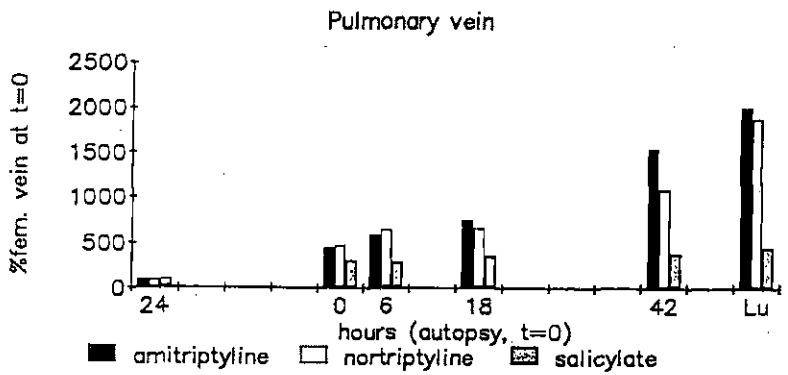

FIGURE 3
Postmortem Changes in Drug Concentrations in the Pulmonary Vein
Lu = lung

these drugs the lung tissue to blood concentration ratio may be as high as 200.[29] This large concentration gradient provides the basis for postmortem drug diffusion into the pulmonary vessels.

Data from an unpublished case study[30] demonstrates the importance of drug diffusion from the lungs. A twenty-nine-year-old male (74kg and 172cm) was found dead about 8 hours after an overdose of amitriptyline and salicylate and came to autopsy 21 hours later. Blood samples were taken at 0, 6, 18, and 42 hours. The initial femoral vein sample contained amitriptyline, nortriptyline, and salicylate at concentrations of 2.5, 0.7, and 81 mg/L. Corresponding drug concentrations for the pulmonary artery at 0 hours were 12, 3,1, and 94; for the pulmonary vein at 0 hours were 11, 3,0, and 244; for the pulmonary artery at 42 hours were 34, 6.5, and 309; for the pulmonary vein at 42 hours were 39, 7.0, and 310; for the femoral vein at 42 hours were 1.0, 0.6, and 86. Drug concentrations in the pulmonary vessels at 42 hours were more than twice that in other blood samples. If the drug concentration in the femoral vein at 0 hours is taken as representative of the concentration in all vessels at the time of death then the changes in the pulmonary vessels can be plotted against time since death (Figure 3). Postmortem elevation of pulmonary vein drug levels occurs earlier than in the artery probably because diffusion through the thinner-walled vein occurs more readily.

An autopsy study of eight methamphetamine abusers[31] demonstrated marked site dependence of the drug with highest concentrations in the pulmonary vein. Methamphetamine concentrations in cardiac blood samples were consistently higher than in femoral samples, and concentrations in left heart blood samples

were 1.9 to 2.6 times higher than those in right heart blood samples. The highest concentrations were found in pulmonary vein samples reflecting diffusion from drug depots in the lung.

It is possible that the rich capillary network of the lungs plays a dual role in postmortem drug redistribution, firstly by providing a conduit for rapid diffusion, and secondly as a drug reservoir following antemortem drug sequestration. The endothelial cells of capillaries heavily concentrate phenobarbital, as demonstrated by immunohistochemical staining[32] and this may be true of other drugs. Postmortem release of drugs from endothelial cells into the blood is potentially very rapid. Certainly endothelial cells are shed into the blood during the first day postmortem.[33]

Drug concentrations in cardiac blood and corresponding femoral blood samples for 121 cases[34] provided an overview of postmortem changes for a diverse range of drugs. The authors highlighted the cardioactive drug diltiazem, a calcium channel blocker, which is concentrated in the myocardium, as an example of how such cardioactive drugs are likely to show postmortem redistribution from the myocardium into cardiac blood. Previously,[4,5] it has been shown that digoxin levels rise in postmortem blood and that release from the myocardium, where the drug concentration is approximately thirty times that of the blood, is the likely mechanism.

Drug diffusion postmortem from solid organs into the blood is not simply a matter of passive diffusion as described by Fick's first law of diffusion, which states that the rate of diffusion is proportional to the concentration gradient across the diffusion barrier. Postmortem there is also some natural movement of blood within the vessels so that there is some physical transport of drugs. These movements are associated with pressure changes resulting from rigor mortis and putrefactive gas formation. The extent of this postmortem blood flux is also influenced by the degree of fluidity of the blood in the individual case. The resultant ebb and flow of blood has been incorrectly described as postmortem "circulation."[35] During the first twenty-four hours postmortem there is little movement of blood other than reflux from the heart into the superior vena cava and the associated neck veins. With the increase in intra-abdominal pressure, there is blood reflux from the abdominal aorta into the thoracic aorta, from the inferior vena cava into the right atrium and contiguous superior vena cava, and reflux from the left cardiac chambers into the pulmonary veins. Up to this point in time there is no appreciable movement of blood in peripheral vessels. With the resolution of rigor mortis, which is the result of muscle putrefaction, the heart chambers are emptied of blood and there is flow into peripheral arteries with associated slight movements of venous blood. Thus the blood in peripheral vessels undergoes the least postmortem flux.[35] An experimental study of postmortem blood movement in rabbits[36] concluded that these changes were also influenced by cardiac and arterial bed contraction, strictly physical (gravitational) phenomena related to cadaver position, and the tendency of blood movements

to occur along the most linear natural anatomical trajectories. Consequently, it is to be expected that blood flux will be highly variable from case to case.

One approach to the problem of postmortem drug changes in blood has been to look for alternative or corroborating tissues for analysis. One suggestion has been to use skeletal muscle. In a preliminary study,[37] drug levels in thigh muscle (not otherwise specified) were compared with levels in aortic blood. In general most of the drugs detected in the blood were also detected in muscle and the concentration ratio between the two specimens was usually near or greater than unity. In those instances where blood concentrations were several fold those in muscle, the suggested explanation was death soon after drug ingestion and prior to equilibration between blood and muscle drug levels. Similarly, the explanation for muscle drug levels greater than blood levels was a lag time between ingestion and death allowing for equilibration and metabolism. Thus the determination of the blood/muscle drug concentration ratio could be of interpretive value in estimating the time of drug ingestion relative to death and an interpreting falsely elevated blood drug levels due to postmortem release. This relationship seemed to be true for propoxyphene and tricyclic antidepressants but exceptions were codeine and cocaine metabolites which were always present in very much lower concentrations in muscle than in blood. The study would have been of greater value had a peripheral blood sample been taken in addition to or instead of the aortic blood sample, but the results remain illuminating.

Many organic compounds are highly lipophilic, a characteristic that permits rapid penetration of cell membranes and uptake by tissues, so that they distribute and concentrate in body fat. These toxicants appear to accumulate in fat by simple physical dissolution in the neutral fats,[28] which constitute about 50 percent of the body weight of an obese individual and about 20 percent of the body weight of a lean athletic individual. Although drugs with extremely high lipid-water partition coefficients have a tendency to accumulate in body fats, their distribution into body fats occurs slowly because blood flow to adipose tissue is low (about 3 mL/100 g/minute). Consequently, equilibration between blood and adipose tissue concentrations of these toxicants occurs slowly and may not have been achieved if death occurs very soon after administration. Examples of such circumstances would include some anaesthetic deaths, and deaths from glue sniffing and petrol sniffing.[38] In such cases there is the potential for postmortem diffusion of the toxicant from the blood into adipose tissue, thus lowering the postmortem blood levels of the toxicant. An animal model of this has been developed.

Postmortem diffusion of toluene after antemortem exposure to the vapor (4000 ppm) for fifteen and sixty minutes was studied in rats killed and left for twelve and twenty-four hours in fresh air.[39] In the rats exposed for fifteen minutes, there was a marked postmortem fall in blood toluene concentration, and a lesser fall in liver and kidney concentration but a marked increase in concentration in subcutaneous and intraperitoneal fat. The changes were similar but less marked in the rats exposed to vapor for sixty minutes prior to sacrifice. Thus short

exposure to the highly lipid soluble volatile resulted in a time-lag effect in toluene distribution to adipose tissue with the result that there was postmortem diffusion from the blood and solid organs into fatty tissue.

Recently it has been suggested that the agonal aspiration of vomitus having at least 0.80% w/v ethanol is associated with an elevation of the aortic blood ethanol concentration when contrasted with right atrial and inferior vena caval blood.[40] In a group of ten cases, the mean ethanol concentration in aortic blood was 238 mg% (range 120 to 390 mg%), in right atrial blood 191 mg% (range 110 to 260 mg%), and in inferior vena cava blood 182 mg% (range 100 to 270 mg%). The mean ethanol concentration in gastric fluid was 1.55% w/v (range 0.83 to 3.10% w/v). Even without agonal vomiting, gastric fluid might enter the airways as a result of postmortem relaxation of the oesophogeal sphincter and passive regurgitation.

A simple experimental model using human cadavers, demonstrated that drug- and ethanol-laden gastric material present in the airways postmortem would allow the diffusion of drugs and ethanol into the blood.[41] An acidified slurry of 60 ml, N/20 HCl, 3% w/v ethanol, 3.25 g paracetamol, and 325 mg dextropropoxyphene, was introduced into the trachea of five cadavers. Blood samples were taken forty-eight hours later. Highest blood ethanol concentrations were found in the pulmonary vein (mean 58 mg%, range 13 to 130) and pulmonary artery (mean 53 mg%, range 10 to 98) but occasionally high concentrations were found in the superior vena cava (maximum 74 mg%) and the aorta (64 mg%). The pattern of concentrations of both dextropropoxyphene and paracetamol mirrored that of ethanol. The fact that concentrations in the aorta were higher than the left heart and concentrations in the superior vena cava were higher than the right heart suggested that there is direct diffusion into both the aorta and superior vena cava. Diffusion into the aorta might be from the immediately adjacent left lung, left bronchus, and carina. Diffusion into the superior vena cava is presumably via veins immediately adjacent to the trachea. The study provides a further reason why the sampling of cardiac blood for toxicological analysis is undesirable.

No specialized system exists in mammals for the sole purpose of absorption of drugs and other toxicants. Few toxicants are actively absorped by the gastrointestinal tract and most enter the body by simple diffusion. Postmortem it is clear that this diffusional absorption of drugs continues, with the exception that there is no active blood circulation to distribute the absorbed drug around the body. In life, the absorption of drugs can take place along the entire gastrointestinal tract, even in the mouth and rectum, and there is no reason to believe that the same cannot occur postmortem. That alcohol can diffuse out of the stomach postmortem to significantly raise the cardiac blood concentration has been known since as long ago as 1949,[42] and there have been many detailed studies since that time.[43] Table 4 shows the ethanol and methanol blood concentrations in samples taken forty-eight hours after the infusion of a 10% v/v solution of alcohols into the stomach of a cadaver.[44]

TABLE 4

Methanol and Ethanol Concentrations (mg/dl) in Blood Samples 48 Hours After Introducing 400 ml of 10% v/v Solution into the Stomach

	Methanol (mg%)	Ethanol (mg%)
FV	14	5
IVC-ir	23	12
PV (right)	38	23
IVC-sr	53	33
Right heart	53	32
SVC	63	43
PA	81	55
Left heart	93	61
Aorta	169	129
PV (left)	251	211
Pericardial fluid	455	401

FV = femoral vein
IVC-ir = inferior vena cava, infrarenal
PV = pulmonary vein
IVC-sr = inferior vena cava, suprarenal
SVC = superior vena cava
PA = pulmonary artery

In cases of drug overdose, there may be large amounts of unabsorbed drug present in the stomach and small bowel at the time of death. This may occur simply because the drug dose taken was so far in excess of the lethal dose that death supervened before absorption was complete. Some drugs, such as aspirin, iron, glutethimide, and ethchlorvynol, tend to form concretions or bezoars which delay absorption. Other drugs, such as barbiturates, delay gastric emptying.[45]

The extent of passive postmortem drug diffusion from a reservoir in the stomach and duodenum has been studied in a rat model.[46] After killing with carbon dioxide, and within ten minutes of death, 75mg amitriptyline was administered to rats by a gastric tube. As early as five hours postmortem, the drug was detected in the liver lobes adjoining the stomach. The drug concentration in the liver increased with time but was significantly lower in the lobes situated furthest away from the stomach. However the rat is a small animal so that the diffusion distances between the different sampling sites are small and it is to be expected that the phenomenon would be more dramatic in the rat than in the human.

The anatomical relationships of the human liver that are relevant to postmortem drug diffusion are primarily those with the esophagus, stomach, pylorus, and proximal duodenum. The greater part of the inferior surface of the left lobe of the liver is in direct contact with the stomach (Figure 4). The gastric pylorus and proximal duodenum rest against the quadrate lobe of the liver, the gallbladder

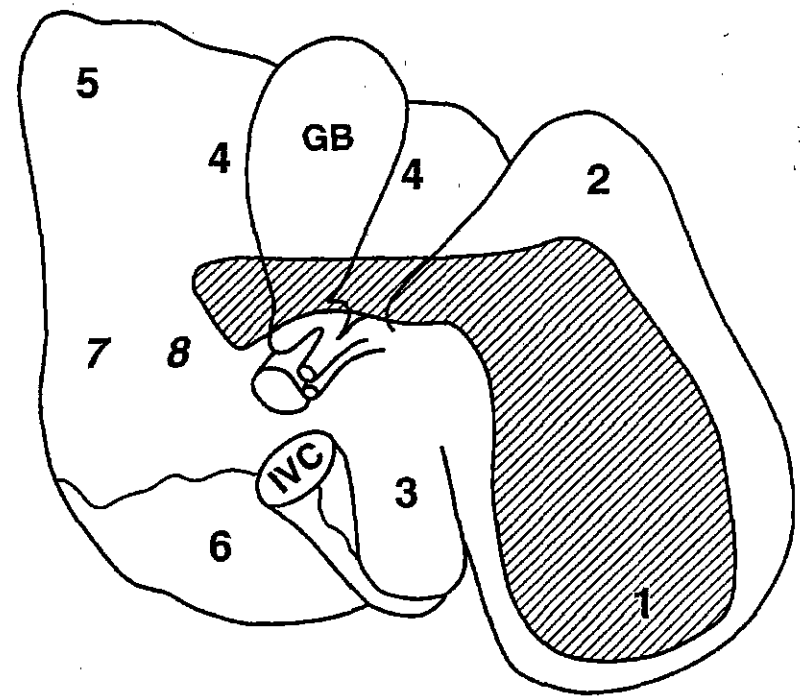

FIGURE 4
Inferior Surface of Liver Showing Gastroduodenal Contact Area (Shaded) and Sampling Sites for Drug Analysis
GB = gallbladder
IVC = inferior vena cava

and a small area of the right lobe immediately adjacent to the gallbladder neck. Clearly there is potential for drugs within the lumen of the esophagus, stomach, and duodenum to diffuse into the substance of the immediately adjacent liver. Equally there is potential for drug concentrated within the bile in the gallbladder to diffuse into the quadrate lobe and the immediately adjacent part of the right lobe. Indeed, it is common at autopsy to see bile staining of the liver adjacent to the gallbladder as a result of postmortem diffusion from the gallbladder. Other visceral areas of the inferior and posterior surfaces of the liver are in contact with the transverse colon, right kidney and right suprarenal gland, but these seem unlikely to have relevance to postmortem drug diffusion.

There is no published human case data demonstrating postmortem diffusion of drug from the stomach into the liver. Data from one unpublished case[47] suggests

that it does occur. Following death from an overdose of zopiclone (a novel nonbenzodiazepine hypnotic agent), eight liver samples were obtained at autopsy ninety-six hours after discovery of the body. The sites of liver sampling are shown in Figure 4 together with the gastroduodenal contact areas of the liver. Zopiclone concentrations in the samples 1 through 8 were, respectively, 3.7, 4.9, 1.9, 3.3, 2.1, 1.8, 1.9, and 1.9 mg/kg. These variations likely reflect diffusion from gastric contents, which comprised 700 ml of fluid and semidigested food with a drug concentration of 55.1 mg/L, as well as from bile in the gallbladder, which had a zopiclone concentration of 14.1 mg/L.

In another animal model of postmortem diffusion from the stomach,[48] rats were given a high oral dose of sodium secobarbital (250 mg/kg) and sacrificed two hours later. Over the following seven days, secobarbital concentrations in blood increased nearly eight fold and in liver and spleen more than ten fold, likely reflecting postmortem diffusion of previously unabsorbed drug in the gastric lumen. Kudo studied postmortem changes of triazolam in rats given 5 mg/kg orally and sacrificed after one hour with tissue and blood samples collected at zero, one, and two days. The mean blood level of triazolam immediately after death was 6.33 ng/g, and the level was increased to 18.2 ng/g one day after death. Concentrations in the abdominal muscle, spleen, liver, and kidney markedly increased and, after two days, reached ten to two hundred times the initial levels. By contrast, thigh muscle and brain levels were constant. The results demonstrated that triazolam in the stomach diffuses through the stomach wall into the abdominal tissues and organs postmortem.

In Japan some alcohol abusers drink thinner solution (toluene, ethyl acetate, and isobutanol) mixed with ethanol. Concern that thinner solution in the stomach might diffuse into the tissues postmortem led researchers to develop a rat experimental model.[49] Animals were killed one hour after oral administration of 1 ml of standard thinner solution and then left at room temperature for forty-eight hours. There was a marked increase in the concentration of toluene in the liver and a gradual increase in the lung, kidney, and abdominal muscles with concentrations reaching three to fifteen times as high as the initial levels. On the other hand, there was only a slight increase in the blood and brain levels and no change in the thigh muscle sample. Isobutanol showed similar patterns of increase to those of toluene. It was concluded that a postmortem diagnosis in human case material should be made on the basis of the analysis of muscle located far from the viscera together with the stomach contents.

As the early postmortem period ends and putrefaction begins, sanguinous fluid accumulates in the pleural cavities and it has been suggested[50] that this putrefactive fluid might prove useful for toxicological analysis. These cases are recognized as presenting both technical and interpretative difficulties, particularly since the major shifts in blood drug concentrations will have been largely completed. Consequently in putrefying bodies, pleural fluid may be as good as, or more accurately as bad as, blood for analytical purposes. When putrefactive fluid

TABLE 5

Drug Concentrations (mg/L) in Putrefactive Fluid Accumulating in the Left and Right Pleural Cavities in Two Cases of Co-proxamol Overdose

	Paracetamol		Dextropropoxyphene	
	Left	*Right*	*Left*	*Right*
Case 1	1045	297	5.7	2.2
Case 2	1669	334	30.3	20.5

is sampled from both the left and right pleural cavities it appears that drug concentrations on the left are consistently higher than on the right (Table 5). The most plausible explanation for this is the diffusion of unabsorbed drug residue from the gastric lumen.[47]

In putrefying bodies the site-dependent drug concentration differences in blood appear to be largely absent.[15] The likely explanation for this is that significant drug release from tissue depots into the blood has already occurred and there has also been sufficient time for diffusion within the vascular tree and for the mixing of blood due to postmortem blood flux. It follows that although blood samples from putrefying bodies may show little or no site-to-site variation in drug concentrations, nevertheless, these blood drug concentrations are likely falsely elevated, a new equilibrium having been attained in the corpse.

In decomposing bodies there is often an absence of blood and an absence or sparcity of solid tissue suitable for toxicological analysis. In this circumstance drugs may be identified through analysis of maggots (fly larvae) feeding on the body. An overview of such cases, listing the fly species, drugs, and human tissue studied, has been published elsewhere.[51] The fundamental premise underlying this technique is that a drug detected in maggots feeding on a body can only have originated from the tissues of the body. While this may be true, it is now clear that the absence of a drug in the maggots is not necessarily an indication that the drug was not present in significant concentrations in the corpse.[52] It appears that maggots have a considerable capacity to eliminate drugs present in their food source and that this ability varies with drug type. Thus far the database on forensic entomo-toxicology is too sparse and unstructured to draw wider conclusions. Ideally drug concentrations should be measured in residual skeletal muscle, the principal food source for fly larvae, as well as in washed maggots. To what extent drugs are retained in successive levels of the food chain is entirely unknown; drugs might be detectable in beetles feeding off fly larvae.[53]

As part of the putrefactive process the potential exists for the degradation of drugs by bacteria. However, publications concerning the stability of drugs during putrefaction are comparatively rare. Some drugs are known to be unstable in mortuo so that postmortem blood concentrations of these drugs have a potential to fall, a change opposite to that produced by diffusional redistribution from

solid organs into blood. This is a somewhat different issue from the stability of drugs in blood and tissue samples stored in the laboratory under refrigeration when putrefaction and enzymatic activity is effectively arrested. Most drugs do not deteriorate in blood samples stored under laboratory refrigeration for up to four months,[54] although some drugs, such as amitriptyline, nortriptyline, and desipramine, may show apparent increases in concentration possibly resulting from improved recovery of protein-bound drug from the sample following protein degradation. One notable exception is chlordiazepoxide, which undergoes considerable deterioration in whole blood samples stored at 4°C but not in serum samples.[54]

The postmortem stability of benzodiazepines has been studied extensively.[55] The prototype of this class of drugs, chlordiazepoxide (Librium) was unstable in spiked postmortem blood at room temperature (25°C) and at 4°C. In a solution originally at 5 mg/L, the drug was not detectable within one to two weeks. A two-step mechanism for the degradation of chlordiazepoxide and norchlordiazepoxide was suggested. The initial step is likely a temperature dependent chemical breakdown of the original compound to demoxepam. The demoxepam may then be converted to nordiazepam by contaminating microorganisms possibly by a reductase enzyme. Both demoxepam and nordiazepam are metabolites of chlordiazepoxide in humans as well as being postmortem breakdown products. Thus the interpretation of chlordiazepoxide and metabolite concentrations in postmortem cases will be difficult when there is a time lag of greater than several days between death and specimen acquisition. An initial toxic chlordiazepoxide concentration of 5 mg/L can rapidly decrease within days to a concentration that could be considered therapeutic.

Others[56] have studied the stability of the benzodiazepines in bacteria contaminated drug-spiked postmortem blood. At room temperature four of the benzodiazepines tested (chlordiazepoxide, chlorazepam, trinitrazepam, and nitrazepam) were completely degraded in six days. Flunitrazepam was completely degraded within about two to three days with the production of 7-amino-flunitrazepam. Since the routine GC-EC analytical method for benzodiazepines is extremely sensitive for flunitrazepam but does not detect 7-amino-flunitrazepam there is a real possibility of a fatal intoxication being missed on routine analysis. As with chlordiazepoxide there is the additional problem that the bacterial degradation product is the same as the principal human metabolite.

One study,[57] examined the stability of fifty-six drugs and drug-related compounds in putrefying human liver macerates. In specimens having a high bacterial contamination from deliberate faecal inoculation, the tissue levels of labile drugs generally fell sharply within three or four days. By contrast, losses of labile drugs from the aqueous solutions used as controls were small. Drug lability to bacterial degradation was related to the presence of one of three chemical structures in the drug:

1. Oxygen bonded to nitrogen but not to carbon or sulphur. This occurs with nitro groups bonded to either an aromatic nucleus or to a nonaromatic structure; with oximes and with N-oxide structures, for example, chlordiazepoxide;
2. Sulphur in a chain bonded as a thiono-group (C = S, P = S), for example, malathion;
3. Aminophenols, that is, OH and NH2 on the same aryl nucleus.

Structures possessing a primary aryl amine group, but which are not phenolic or are phenolic but do not possess such an amine group, or have a substituted amine group, for example, paracetamol, are all stable. The stability of other drugs reflected the stability of chemical structures in which carbon bonds with oxygen and nitrogen, nitrogen bonds with hydrogen, and sulphur bonds with oxygen. Sulphur, forming part of a heterocyclic ring causes some instability to putrefaction, for example, dothiepin and the phenothiazines. The variability of degradation of these latter drugs suggests that the bacteria capable of degrading them are less widely encountered than those capable of breaking down the other labile chemical structures. While the lability or stability of a drug to putrefactive bacterial degradation may be generally inferred from its chemical structure, anomalies have been observed, for example, thiopentone would be expected to be labile but is stable, and bendrofluazide would be expected to be stable but is unstable.

The stability of cephalosporins was studied at temperatures ranging from −20°C to 55°C in a rat model.[58] Cefoperazone in solution in water showed a temperature dependent degradation that was minimal at ten days and 10°C but 100 percent at one day and 55°C. Comparable degrees of drug degradation were seen in blood and tissue (muscle, liver, kidneys, and lungs) samples from rats who had been given the drug intravenously and the samples taken immediately after killing. By contrast, in rats that were killed and left intact at room temperature (ca.25°C) for up to five days, drug degradation in the tissues was not so marked. The authors suggested that this was the result of protection of the drug, in the intact cadaver, from exposure to the air. Whether or not this is the explanation, the experiment illustrates that drug stability experiments conducted in vitro may not be directly comparable with intact cadavers.

In a series of case studies, cocaine showed site-dependent variation in postmortem blood concentrations.[59] Cocaine levels in aortic and cardiac blood were generally higher than in peripheral blood in keeping with postmortem diffusion from tissue depots. However subclavian blood samples frequently showed a fall in cocaine levels over time, suggesting that postmortem hydrolysis had more than compensated for any diffusional redistribution effect (Table 6). These observations emphasise the danger inherent in attempting to use postmortem blood cocaine levels to determine if the deceased was under the drug's influence at the time of a fatal incident, such as a motor vehicle accident or a homicide.

TABLE 6

Cocaine and Benzoylecgonine Concentrations (mg/L) in a 22-year-old Black Male Who Suddenly Collapsed During Cocaine-Excited Delirium; Scene to Autopsy Interval Was 20 Hours (18 Hours for Vitreous Humour) (Abstracted from Case 2, Table 2, Reference 59)

Sample	Cocaine	Benzoylecgonine
Scene of Death		
subclavian v.	2.3	3.7
femoral v.	1.8	3.6
vitreous	1.0	1.1
Autopsy		
subclavian v.	1.2	2.8
femoral v.	3.9	8.1
vitreous	3.5	1.7

The competing effects of postmortem drug redistribution and drug degradation are illustrated by a detailed case study[60] involving an overdose of tranylcypromine (TCP), an antidepressant structurally like amphetamine. TCP has a relatively low Vd ranging from 1.11 to 5.68 L/kg and as might be predicted, showed a *moderate postmortem* redistribution phenomenon with concentrations lowest in peripheral blood (0.17 mg/L) at zero hours and highest in central vessels at twenty-four hours (0.52 mg/L) after starting the autopsy. However, at seventy-two hours blood TCP concentrations fell below those at zero time but these samples showed marked putrefactive changes. Control blood samples spiked with TCP and incubated for forty-eight hours at 37°C showed a 58 percent fall in drug concentration, suggesting that the in mortuo changes reflected drug degradation. Fortuitously, lithium was also present and this drug, which has a small Vd (0.8 L/kg) and is chemically stable, did not show a rise-and-fall pattern of change in blood concentration. Thus the nett TCP blood concentration reflected the early dominance of the diffusional effect of postmortem redistribution and the later dominance of putrefaction-related degradation.

One of the most fundamental questions of postmortem forensic toxicology is: "How much drug did the decedent take?" Traditionally the answer has been inferred from comparison of blood drug concentrations with published case reports of fatal intoxications in which the amount of ingested drug was known and from reports in the clinical literature. In recent years, pharmacokinetic equations have been used increasingly despite the limitations of the method.[15] Regardless of all other factors, attempts at pharmacokinetic estimations of dose are dependent upon whether drug concentrations measured in autopsy blood specimens accurately reflect the concentration of the drug at the time of death.

The difficulty of calculating the drug dose taken from the postmortem drug concentration in a blood sample, often of unknown provenance, is well illustrated with propoxyphene, an abused narcotic. Therapeutic blood levels after single doses of up to 195 mg (six tablets) fall within the range 0.06 to 0.75 mg/L and a fatal dose has been estimated at forty tablets, but perhaps as low as fifteen to twenty tablets if taken with alcohol. A series of thirty fatalities had postmortem blood concentrations ranging from 1.3 to 24.4 mg/L.[61] The higher level of postmortem blood propoxyphene concentration would suggest an ingested dose which is hardly credible and must reflect artefactual postmortem elevation.

In the early 1970s, prior to the appreciation of postmortem drug redistribution artefacts, some case fatalities were reported with blood concentrations of propoxyphene greater than 10 mg/L and this was sometimes attributed to analytical shortcomings.[62] It became apparent later that about 10 percent of fatal poisonings with propoxyphene had blood concentrations exceeding 10 mg/L.[63] One case, reported in some detail,[64] illustrates the problem well: a white male (77 kg and 185 cm) died from an oral overdose of Darvocet-N (propoxyphene napsylate 100 mg and acetaminophen 650 mg; dextropropoxyphene napsylate 100 mg is approximately equivalent to 66 mg of dextropropoxyphene hydrochloride). Blood propoxyphene was 87.8 mg/L, norpropoxyphene 8.8 mg/L, and acetaminophen 172 mg/L. The authors reasoned that the decedent's body contained 6 litres of blood so that the concentrations of propoxyphene and norpropoxyphene found would account for about nine Darvocet-N tablets suggesting that he had had access to more than the twelve tablets legally prescribed. The conclusion may be correct; however, estimates of the number of tablets taken using standard Vd calculations for propoxyphene and acetaminophen are over 1000 and 20, respectively. The disparity between the two calculations is so great and the estimated propoxyphene dose so extreme as to leave little doubt in retrospect that the analytical result reflects artefact from postmortem drug redistribution.

A review of concentration differences for propoxyphene between femoral and heart blood samples was made in thirty-four cases of propoxyphene poisoning.[65] In five of the thirty-four cases, the femoral blood concentration was less than 1 mg/L but the heart blood concentration was greater than 1 mg/L. For the five cases the mean femoral blood concentration was 0.59 mg/L (range 0.40 to 0.79 mg/L) and the heart blood concentration mean was 4.3 mg/L (range 1.4 to 8.8 mg/L) with the mean heart/femoral blood concentration ratio 7.4 (range 2.9 to 13.3). It was concluded that the published data on fatal propoxyphene blood concentrations can be misleading if a femoral blood concentration from an individual case is interpreted against this data. The authors pointed out that in equivocal cases there is a serious risk of misinterpretation of the cause and manner of death if a single site blood analysis is made. Some have claimed[66] that after acute overdosage with propoxyphene, the liver concentrations of the drug and its metabolite exceed the respective blood concentrations by a factor of 10 or more, making it possible to distinguish this situation from chronic therapeutic administration, in

TABLE 7

Body Load of Propoxyphene and Paracetamol in Three Co-proxamol Overdoses, Calculated by Addition of Organ/Tissue Loads and by Vd, and from Circumstantial Evidence

	Body drug load (mg)					
	By organ wt.		By Vd		Circumstantial	
Case	*PX*	*Para*	*PX*	*Para*	*PX*	*Para*
1	291	10389	2898	24778	2600	26000
2	159	8354	2432	14084	1625–1950	16250–19500
3	123	6354	3042	10190	1235	12350

PX = propoxyphene
Para = paracetamol (acetaminophen)

which liver concentrations are of the same order of magnitude as those in the blood. Other data indicate that liver:blood concentration ratios are very much influenced by blood sampling site and that liver concentration can be very close to that in peripheral blood in a clear case of overdose.[17]

For a drug with a low Vd, which consequently does not show postmortem drug redistribution, calculation of the ingested dose from pharmacokinetic formulae may be reasonably accurate. This seems to be true for paracetamol, which has a Vd of approximately 1 L/kg. When the ingested drug is co-proxamol, a compounded preparation of paracetamol and dextropropoxyphene, the dose of the effective lethal agent, dextropropoxyphene, can be derived from the calculated dose of paracetamol, given the fixed ratios of the drugs in the compounded preparation. Table 7 shows the calculated drug doses in three fatalities from co-proxamol.[67] One calculation was by addition of the individual tissue/organ drug loads. For the major viscera, the organ drug load can be calculated accurately from the known drug concentration and the organ weight taken at autopsy. Drug load in blood can be calculated from the peripheral blood drug concentration (the lowest concentration) and the blood volume estimated from a published nomogram.[68] Drug load in skeletal muscle can be estimated on the assumption that for a 70 kg ideal body weight person the skeletal muscle mass is 30 kg. Body paracetamol load was underestimated by this organ weight calculation relative to the Vd calculation, but the latter was only a minimal underestimate of the suspected ingested dose, particularly when allowance was made for the known quantity of unabsorbed drug in the stomach. By contrast, body dextropropoxyphene load was seriously underestimated by the organ weight calculation, which, however, does not include the drug load in body fat or the metabolite load. Body propoxyphene calculated by Vd overestimates by up to 2.5 times the suspected ingested dose, the error increasing with decreasing dose.[67] However

the propoxyphene dose can be inferred from the Vd calculation of the paracetamol dose.

All that has been said thus far relates to experimental studies or fatalities in adults and any extrapolation to fatalities in children should be made with caution. There are important pharmacokinetic differences between children and adults which influence drug toxicity.[69] The important factors are:

1. children have smaller lipid compartments than adults, so that lipid-soluble drugs (for example, tricyclic antidepressants and most other psychotrophic drugs) are not taken up and stored in inactive lipid sites to the extent in children that they are in adults;
2. there are differences in the degree of drug-binding to plasma albumen between children and adults, so that children have a higher ratio of unbound, pharmacologically active drug for a given blood drug concentration; and
3. the ratio of liver weight to total body weight is greater in the child than the adult, being 50 percent higher at two years and 30 percent higher at six years of age.

Excepting newborns, the rate of drug biotransformation in the liver in children is generally enhanced. For example, the imipramine:desmethylimipramine ratio in plasma is approximately 1:3 in children but nearer unity for adults at therapeutic dosage.

In conclusion, the site-dependent and temporal changes in blood and tissue drug concentrations that occur postmortem result from many interacting factors which are difficult to assess in an individual case, although there is an increasing understanding of their general nature. For interpretive purposes, the ideal toxicological sample is a peripheral blood specimen obtained from a ligated vessel immediately after death. All autopsy samples fall short of this ideal, but the more they do so the more contentious will be the interpretation of the analytical results. The problem is made more difficult because an awareness of the phenomenon of postmortem drug redistribution has undermined the reference value of databases of drug concentrations in postmortem blood where the site of origin of the sample is unknown. Given these difficulties, the final interpretation must depend on a consideration of not only the absolute drug concentrations in the postmortem tissues and fluids, but also on their relative concentrations and, above all, on the autopsy findings and circumstances surrounding the death.

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